FEBS Letters 580 (2006) 6489–6495

Nek7 kinase is enriched at the centrosome, and is required for proper spindle assembly and mitotic progression

Nissan Yissachar, Hagit Salem, Tamar Tennenbaum, Benny Motro*

The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel

Received 6 September 2006; revised 27 October 2006; accepted 30 October 2006

Available online 7 November 2006

Edited by Ulrike Kutay

Abstract **Members of the NIMA-related kinases (NRK) family are recently emerging as central regulators of various aspects of the cell cycle. However, the cellular roles of the mammalian NRK, Nek7, remain obscure. We show here that the endogenous Nek7 protein is enriched at the centrosome in a microtubule-independent manner. Overexpression of *wt* or kinase-defective Nek7 resulted in cells of rounder appearance, and higher proportions of multinuclear and apoptotic cells. Down-regulation of Nek7 using a small interfering RNA approach resulted in a significant increase in mitotic cells presenting multipolar spindle phenotype. These results suggest a role for Nek7 in regulating proper spindle assembly and mitotic progression.**

Keywords: NIMA; Kinase; Centrosome; Multipolar spindle; Chromosome segregation

1. Introduction

The mammalian NIMA-related kinase (NRK) family constitutes of 11 structurally conserved proteins, denoted Nek1-11 [1]. The founder kinase, never in mitosis, gene A (NIMA), was originally identified as a central mitotic regulator in the filamentous fungus *Aspergillus nidulans*. Its documented tasks include involvement in chromatin condensation (presumably by phosphorylating histone H3), enabling nuclear entrance of Cdc2/cyclin B complex into the nucleus, and contribution to the dramatic reorganization of the microtubule network seen during mitosis [1,2]. NIMA-like mitotic activities have recently been ascribed to NRKs from protists, protostomes and vertebrates suggesting conservation of these pathways.

The members of the Nek6/7 subgroup are the smallest NRKs, and are consist of a core kinase domain and a short N-terminal tail [3]. Interestingly, this subfamily is remarkably conserved, and the kinase domain of the murine Nek6 and Nek7 are about 76% identical to the *C. elegans* ortholog. Nek6 and Nek7 are quite highly similar to each other (87% identity in the kinase domain), and our analysis suggests that they are expressed in tissue-specific complimentary patterns during embryonic development and in adults [4]. Nek6 and Nek7 has been shown to interact with another Nek kinase, designated Nercc1/Nek9 [5]. Overexpression of a kinase-dead Nercc1 construct prohibits cell cycle progression, and injection of antibodies against Nercc1 results in prometaphase arrest, abnormal spindle dynamics, chromosomal missegregation and aneuploidy [5]. It has been recently demonstrated that activated Nercc1 is recruited to the centrosome during early mitosis, and that depletion of Nercc1 in *Xenopus* egg extracts disrupts the assembly of normal mitotic spindle [6]. Nercc1 phosphorylates Nek6 and Nek7 in vitro, concomitant with increase in their enzymatic activity, and Nek6 levels and kinase activity were shown to be elevated in mitotic cells [7]. Overexpression of inactive Nek6 kinase, or depletion of Nek6 by small interfering RNA (siRNA), arrested the cells in metaphase, and subsequently the cells underwent apoptosis [8]. However, no specific alteration in microtubule dependent structures has been noticed.

To gain insight into the cellular functions of Nek7, we analyzed the subcellular localization of the endogenous Nek7 protein, and manipulated Nek7 levels in culture cells. Our observations indicate that Nek7 is a centrosomal kinase, which regulates proper spindle assembly and mitotic progression.

2. Materials and methods

2.1. Generation of polyclonal anti-Nek7 antibodies

A fragment of Nek7 (a.a. 1–146), containing the N-terminal tail and part of the kinase domain was cloned into pQE expression vector (Qiagen,Valencia, CA) downstream to the 6XHis tag. Purified proteins were injected into rabbits, and the resulting antibodies were affinity-purified using the corresponding Nek7 fragment coupled to an activated cyanogen bromide Sepharose column (Sigma).

2.2. DNA transfections and siRNA tretments

The cloning of HA-tagged Nek7 and Nek6 mammalian expression vectors has been described before [3]. To create the Ad-HA-Nek7 vector, the HA-tagged full-length Nek7 sequence was cloned into the SwaI site of the pAxCAwt adenovirus 5 vector [9], and viruses were produced in HEK293 cells as described in [9]. HeLa and HEK293 cells were transiently transfected using the calcium phosphate precipitation method [10]. The sequence of the siRNA oligos were as follows: Nek7-siRNA 5′-GCAACUCAACCAUCCAAAUdTdT-3′ (283–301 of mouse *nek7*, [3]), and the control scrambled-siRNA 5′-GCACUAACCUACCAACAAUdTdT-3′ (Invitrogen). The Sequence of Nek7-RNAi inserted into the pSuper vector was 5′-TGGATGCCAAAGCACGTGC-3′ (234–252 of mouse *nek7*), and RNAi against Luciferase was used as a control. The Nek7 siRNA sequences are also identical to the human *nek7* mRNA. The siRNAs were transfected using LipofectAMINE 2000 (Invitrogen) according to the manufacturer's protocol. Western blotting using total cell lysates was performed 48 and 72 h post transfection.

*Corresponding author. Fax: +972 3 535 1824.
E-mail address: motro@mail.biu.ac.il (B. Motro).

Abbreviations: DN, dominant negative; GFP, green fluorescence protein; NIMA, never in mitosis, gene A; NRK, NIMA-related kinase; siRNA, small interfering RNA

0014-5793/$32.00
doi:10.1016/j.febslet.2006.10.069

2.3. Immunofluorescence staining

For immunofluorescence microscopy, cultures grown on coverslips were fixed in 4% paraformaldehyde for 20 min., blocked with 3% BSA/0.5% Triton X-100 in PBS, and incubated overnight with primary antibodies at 4 °C. Primary antibodies were used as follows: the immunized anti-Nek7 serum diluted 1:750, Myc-monoclonal antibodies (Zymed Laboratories, CA) 1:250, HA-monoclonal antibodies (BAbCO, Richmond, CA) 1:1000, and mouse α/β Tubulin (Biomeda, Foster City, CA) 1:1000. Following washes, the coverslips were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-mouse and/or Rhodamin-conjugated anti-rabbit secondary antibodies (Jackson Immuno-Research) (1:150). DNA staining was done with propidium–iodide solution, and the coverslips were mounted on slides with Vectashield mounting solution (Vector, Burlingame, CA) and sealed. Staining was analyzed using an MRC 1024 confocal laser microscope (Bio-Rad).

2.4. Centrosome purification

Centrosomes were isolated as described by Zhou et al. [11]. Briefly, exponentially growing HeLa cells were incubated with cytochalasin D and nocodazole for 1 h. Cells were then washed with 1× PBS, 8% sucrose in 0.1× PBS, and 8% sucrose in water, and incubated with shaking at 4 °C for 10 min in lysis buffer. The insolubles were removed by centrifugation at $2500 \times g$ for 10 min. HEPES was adjusted to 10 mM and DNase I was added to 2 U/ml. After 30 min, lysates were underlain with 60% sucrose solution, and centrosomes were sedimented into the sucrose cushion by centrifugation at $10000 \times g$ for 30 min. This crude centrosome preparation was further purified by discontinuous sucrose gradient centrifugation at $120000 \times g$ for 1 h. Fractions were collected from the top, diluted in PIPES buffer, and sedimented by centrifugation at $25000 \times g$ for 30 min. Pellet protein was resuspended and denatured in SDS sample buffer.

2.5. Immunoprecipitation and in vitro kinase assay

WT or DN-Nek7 were immunoprecipitated from transfected HEK293 cells using anti-HA (BAbCO) or anti-Myc (Zymed Laboratories) monoclonal antibody, respectively. The kinase assay was performed according to Yin et al. [8].

3. Results

3.1. The endogenous Nek7 protein is enriched at the centrosome of interphase and mitotic cells

To examine the subcellular localization of Nek7 in mammalian cells, we raised polyclonal antibodies against the N-terminal region of mouse Nek7 protein (a.a. 1–146). The immunized serum recognized overexpressed Nek7, but not overexpressed Nek6 protein (Fig. 1A), confirming the specificity of the antibodies. Both the immunized anti-Nek7 sera and the affinity-purified anti-Nek7 antibody specifically recognize Nek7 protein in Western blot analysis of various mammalian cell lines (see below, and not shown). Immunocytochemistry staining of HeLaSS6 cells using the immunized anti-Nek7 serum, but not the pre-immune serum, revealed enrichment of Nek7 protein at the centrosome of both interphase and mitotic cells (Fig. 1B). To confirm the centrosomal staining specificity, the anti-Nek7 serum was pre-incubated with NEK7 protein. As can be seen in Fig. 1B, the centrosomal staining was abolished, while cytoplasmic background staining was still observed. Nek7 was clearly detected at the spindle poles at all stages of mitosis, but in contrast to Nercc1/Nek9 [6], it was not associated with the chromosomes following the metaphase-to-anaphase transition, and it was not found in the midbody or spindle midzone at telophase or cytokinesis stages, respectively (Fig. 1B). To specifically decorate the centrosomes, the centriolar marker green fluorescence protein (GFP)-centrin 2 [12] was stably transfected into the HeLaSS6 cell line. Immunocytochemistry staining of these cells demonstrated an overlap between Nek7 and centrin 2 staining. The proximity to the centrioles suggests that Nek7 is confined to the pericentriolar region of the centrosome (Fig. 1C).

To examine whether Nek7's recruitment to the centrosome is dependent on the microtubule network, HeLaSS6 cells were treated with 10 μg/ml nocodazole for 15 min, prior to fixation. Immunocytochemistry staining using anti-α-tubulin antibodies demonstrated depolymerization of the microtubule network, and disassembly of the mitotic spindle in dividing cells. Co-staining of Nek7 and α-tubulin in these cells demonstrated a centrosomal localization for Nek7, independently of the microtubule network and mitotic spindle integrity (Fig. 1D).

To further explore the apparent Nek7 localization to the centrosome, enrichment for centrosomes was carried out using discontinuous sucrose gradient fractionation [11]. The pooled fractions were examined for the presence of the centrosome-associated cytoskeletal protein γ-tubulin, and for Nek7. As can be seen in Fig. 1E high enrichment of γ-tubulin was evident in fractions 6 to 11, and Nek7 enrichment closely paralleled that of γ-tubulin. As expected, no labeling of the fractions was observed using the nuclear marker, pRB (Fig. 1E). Interestingly, while in whole cell extracts Nek7 antibodies mainly stained a protein of about 36 kDa, and very faintly a band of about 38 kDa (presumably phosphorylated and activated Nek7 protein, [7]), in the centrosomal-enriched fractions the higher band was much more prominent, counting for about 35% of Nek7 protein (Fig. 1E).

3.2. Overexpression of wt or kinase-dead Nek7 in culture cells results in multinucleated cells

In order to achieve an efficient overexpression of Nek7 protein in cells, recombinant adenovirus expressing full length Nek7 fused to HA tag was generated according to Miyake et al. [9]. HeLa and NIH3T3 infected cells expressed an HA-tagged protein of the expected size (Fig. 2I). HeLa cells infected with Ad-HA-Nek7 had rounder appearance, and bear much smaller cytoplasmic extensions (Fig. 2II D–F), compared to uninfected HeLa cells, or to cells infected with adenovirus overexpressing a β-gal protein (Fig. 2II A–C). There was higher tendency of the Nek7 infected cells to become multinucleated (1.3% of the cells compared to 0.6% in the β-gal infected cells; Fig. 2II G–I, yellow arrow and Fig. 2IV). In addition, by 48 h post infection, nuclear and cytoskeleton staining revealed a higher proportion of apoptotic cells (18% of the Nek7 infected cells compared to 3.8% of the β-gal infected cells, Fig. 2II G–H, white arrow; Fig. 2III). However, it is not clear at this stage whether the apoptosis was directly induced by Nek7, or (more plausibly) was a late response to induction of spindle assembly checkpoint and mitotic delay [13].

We next examined the cellular consequences of overexpression of a kinase defective construct of Nek7 protein. Nek7 kinase activity was abolished by substituting the invariable glycine residue at the kinase sub-domain I with arginine (designated $Nek7^{G43R}$) (Fig. 2V). In similarity to cells overexpressing Ad-HA-Nek7, multinuclear cells prevail in HeLa cells transiently overexpressing HA-$Nek7^{G43R}$. About 12% of the transfected cells (32 out of 253 cells) had two or more nuclei, compared to 3.3% of the GFP transfected cells (24 out of 722 cells). In addition, an increase in the percentage of the apoptotic cells from 4.1% to 7.5% was observed (30 out of 722 of the GFP transfected cells, compared to 19 out of 253 HA-$Nek7^{G43R}$ transfected cells).

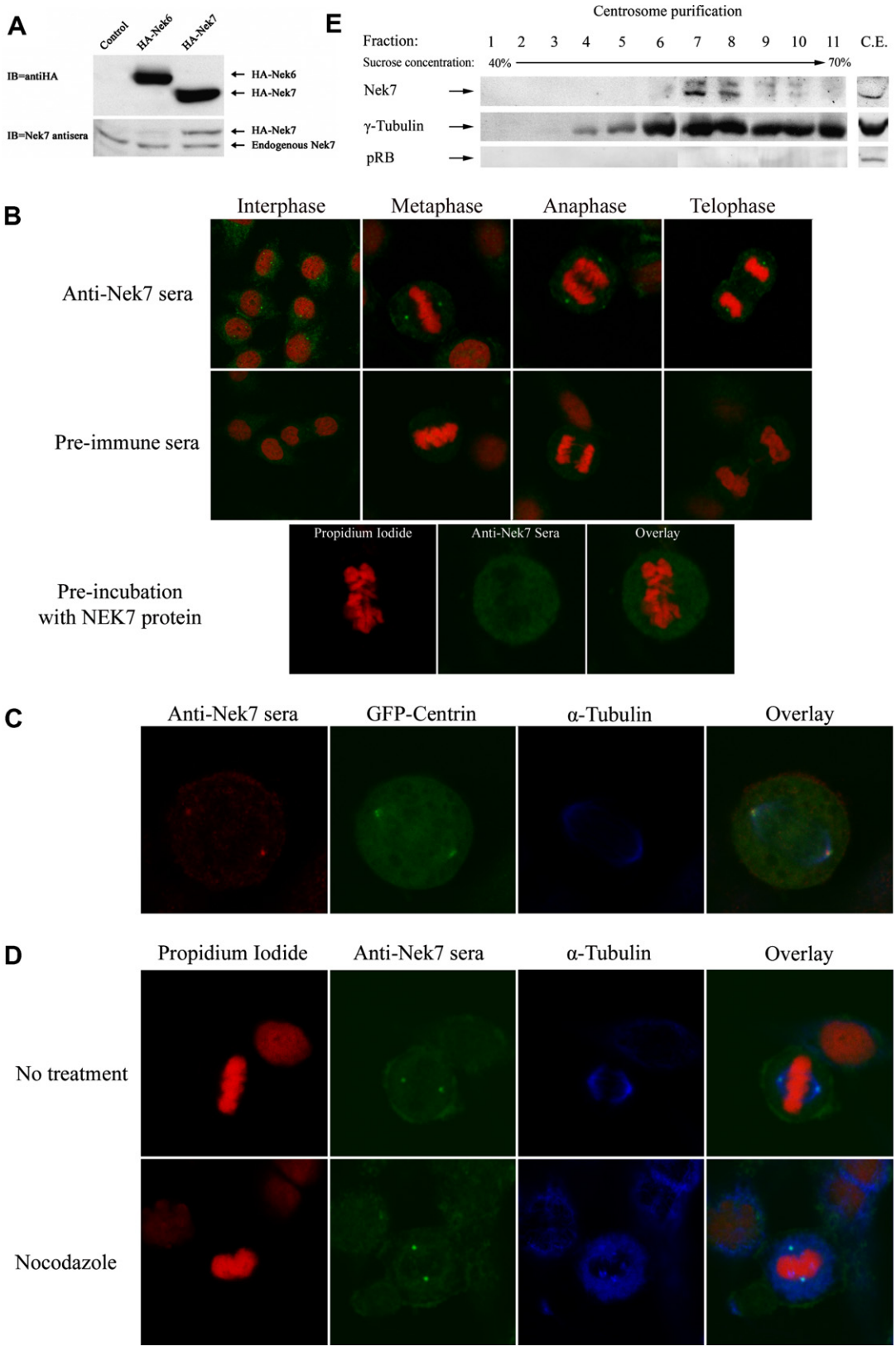

Fig. 1. Nek7 is enriched at the centrosome. (A) Nek7 immunized serum recognized overexpressed Nek7 but not overexpressed Nek6 protein. (B) Nek7 immunized serum, but not the pre-immune serum, detects Nek7 at the centrosome of interphase, metaphase, anaphase and telophase HeLaSS6 cells. Pre-incubation with NEK7 protein eliminated the centrosomal staining. Red: Chromatin (propidium iodide); Green: Nek7 (C) Co-localization of Nek7 and GFP-Centrin 2 to the centrosome of HeLaSS6 cells. Red: Nek7; Green: GFP-Centrin 2; Cyan: α/β-Tubulin. (D) Nek7 localization in HeLaSS6 cells treated with nocodazole. Nek7 localization to the centrosome is not dependent on the microtubule network. (E) Immunoblot analysis of the fractions from the final non-continuous sucrose gradient of the centrosome preparation. The membrane was blotted with affinity purified anti-Nek7 antibodies and re-blotted with anti-γ-tubulin and anti-retinoblastoma (pRB) antibodies.

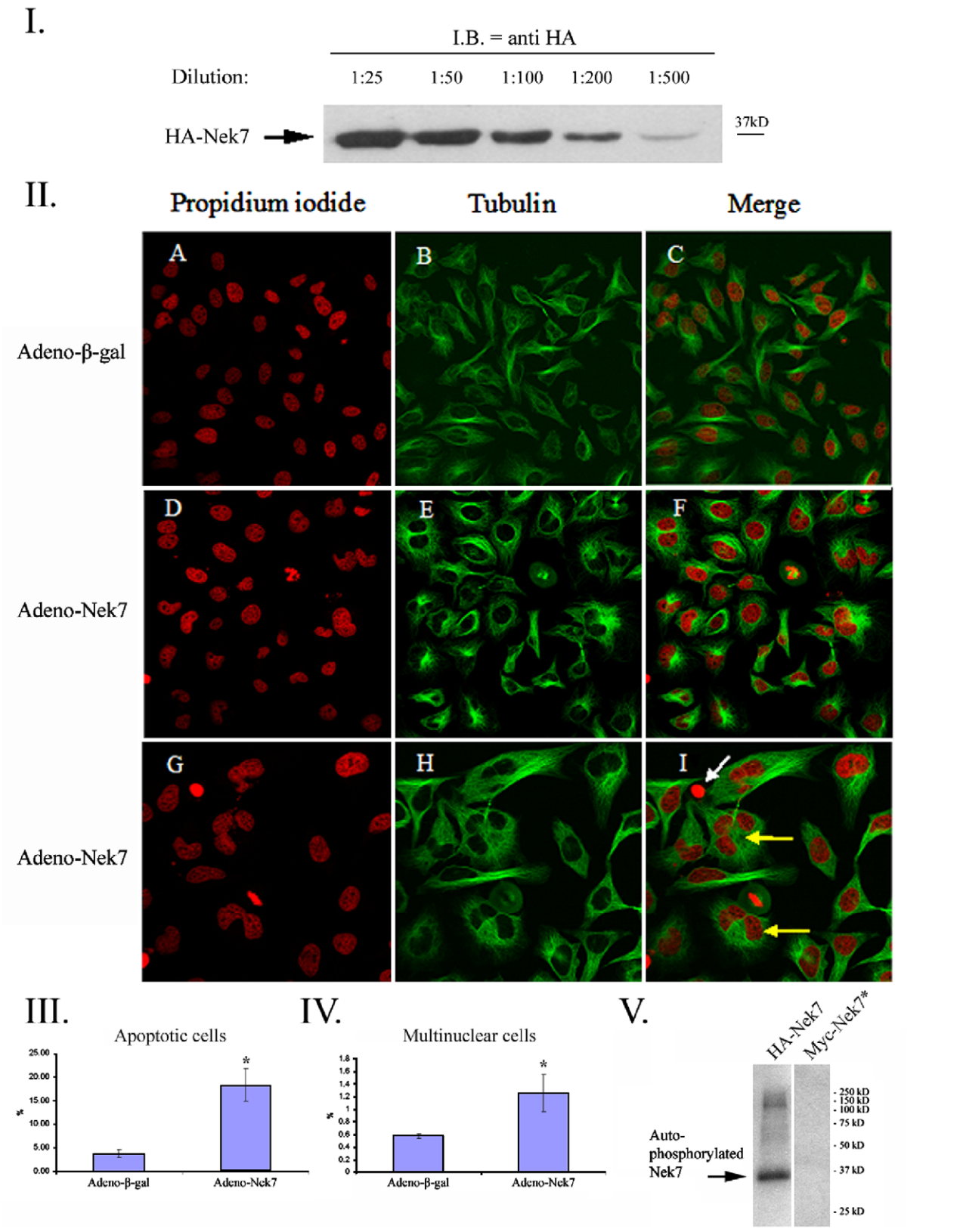

Fig. 2. Ad-HA-Nek7 overexpression in HeLa cells. (I) HeLa cells were infected with supernatant from infected HEK293 cells diluted as indicated. HeLa cell extracts were subjected to immunobloting using anti-HA antibodies. (II) Phenotypic analysis of HeLa cells infected with Ad-HA-Nek7. (A–C) HeLa cells infected with Ad-β Gal (as a negative control), showing the normal HeLa cells morphology. (D–I) HeLa cells infected with Ad-HA-Nek7. The phenotypes include round cells (F), multinuclear cells (yellow arrows in I) and condensed chromatin (white arrows in I). Red: Propidium iodide DNA staining. Green: Immunostaining using α-Tubulin Ab's. (III and IV) Apoptotic (III) and multinuclear (IV) cells following Ad-HA-Nek7 overexpression. Data are based on three independent experiments. $^{*}P < 0.05$, Ad-HA-Nek7 vs. Ad-β Gal. (V) wt or DN-Nek7 were overexpressed, immuoprecipitated and incubated in vitro in a kinase assay buffer containing $P^{32} - \gamma$ATP.

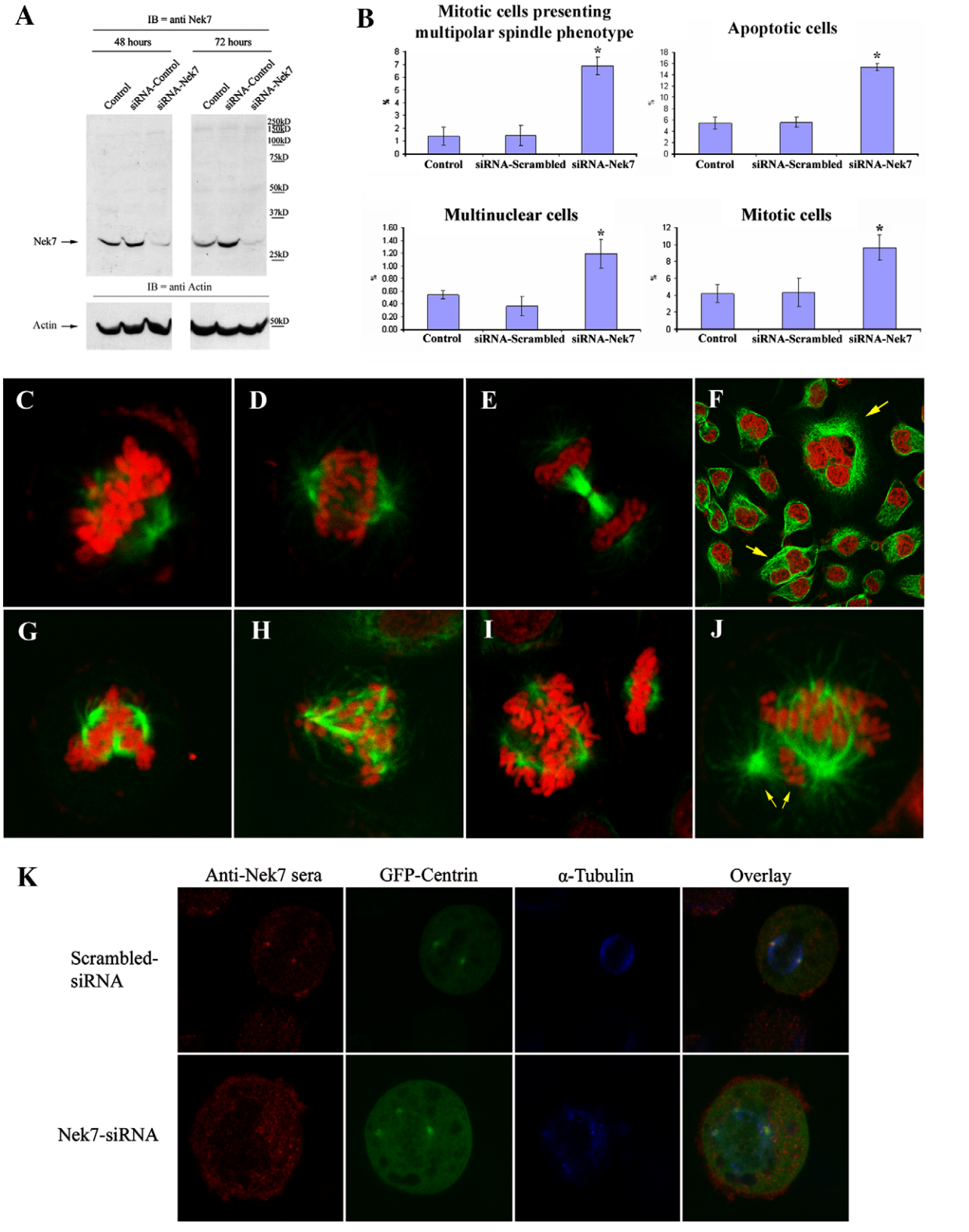

Fig. 3. Knock-down of Nek7 in HeLaSS6 cells resulted in higher incidence of multinuclear cells and mitotic defects. (A) Nek7 levels following transfection with Nek7-siRNA or non-specific control-siRNA, in cells harvested 48 and 72 h post transfection. (B) Statistic analysis of siRNA-Nek7 treated cells exhibiting the indicated phenotypes. Data are based on four independent experiments. $^{*}P < 0.05$ for both siRNA-Nek7 vs. siRNA-Scrambled and vs. control. (C–E) Metaphase, anaphase and telophase (respectively), in HeLaSS6 cells transfected with control-siRNA. (F–J) HeLaSS6 cells transfected with Nek7-siRNA present higher prevalence of multinucleated cells (F, yellow arrow) and abnormal mitotic cells with multipolar spindle (G–J). Red: Chromatin (propidium iodide); Green: α/β-Tubulin. (K) Nek7 centrosomal staining disappeared following siRNA-Nek7 treatment. Red: Nek7; Green: GFP-Centrin 2; Cyan: α/β-Tubulin.

3.3. Knock-down of Nek7 levels results in various mitotic defects

To further investigate the cellular consequences of down regulation of Nek7 pathway, we reduced Nek7 RNA levels by employing the siRNA approach. A 19nt siRNA specific to Nek7 coding sequence (283–301 in *nek7* sequence, [3]) was chosen to target Nek7, and a scrambled siRNA was used as a non-specific control. Transfection of Nek7 siRNA (but not the control siRNA) into HeLaSS6 cells reduced Nek7 protein levels to about 10% of those in untreated cells (in cells harvested 72 h post transfection, Fig. 3A). In the Nek7-siRNA treated cells, but not in the scrambled-siRNA treated cells, the centrosomal staining disappeared, confirming the specificity of Nek7 centrosomal staining (Fig. 3K).

The Nek7 siRNA treated cells displayed distinct phenotypes: First, in comparison to the control siRNA transfected cells, a two fold increase in the mitotic index was observed (Fig. 3B). This may suggest a mitotic delay or arrest of the cells treated with Nek7 siRNA. Second, about 7% of the total mitotic cells displayed multipolar spindle phenotype with three or more spindle poles (compared with about 1% in the control siRNA transfected cells) (Fig. 3B). These cells exhibited abnormal spindle assembly and chromosomes alignment in the metaphase stage, and asymmetric chromosomes segregation was observed in cells proceeding to anaphase (Fig. 3G–J). Similarly to the *wt* or DN-Nek7 overexpression phenotype, treatment with Nek7 siRNA resulted in a three folds increase in the number of multinucleated cells, compared to the control siRNA treated cells (Fig. 3B and F). In addition, nuclear and cytoskeleton staining revealed a three fold increase in the number of apoptotic cells (Fig. 3B). Similar results were obtained using a second RNAi molecule cloned into the pSuper vector, and targeted against different nek7 sequence (not shown).

4. Discussion

Multiplication and structural diversification of the primordial NRK protein created eleven mammalian Nek kinases. Recently, the centrosome, the basal body and the mitotic spindle are emerging as the major and most conserved targets of the NRKs. The current study suggests for the first time that members of the highly-conserved Nek6/7 NRK subfamily are directly involved in centrosome functions. Immunofluorescence staining revealed Nek7 localization to the centrosome throughout all phases of the cell cycle, suggesting that Nek7 is a residential centrosomal component. Mammalian and *Dictyostelium* Nek2 proteins have also been shown to be permanently localized to the centrosome. In contrast, NIMA and *S. pombe* Fin1 proteins localization to the spindle pole body is restricted to mitosis [14,15]. Interestingly, the activated form of Nek6/7-interacting kinase, Nercc1, is specifically localized to the centrosome (and spindle poles) only during mitosis [6]. However, it is not clear, whether cytoplasmic Nercc1 is phosphorylated in prophase and then recruited to the centrosome, or alternatively it is a residential centrosomal protein and is activated during early mitosis [6]. Intriguingly, similarly to Nercc1, the centrosomal Nek7 fraction is highly enriched for the phosphorylated (and presumably activated) Nek7 form. As Nercc1 kinase acts upstream of Nek6/7 [7], it is plausible that activation of Nercc1 at the centrosome is followed by Nek7 phosphorylation.

The most prominent phenotype of Nek7-siRNA-transfected cells is the creation of multipolar spindles. This phenotype could arise through two different major routes: Nek7 could play a role in the synchronization of the centrosome duplication cycle with the cell division cycle, for example through participation in the cdk2 – nucleophosmin pathway [16–18]. Alternatively, Nek7 could be involved in coordinating cytokinesis. Nek7 deficiency will thus results in cytokinesis failure and accumulation of cells with high centrosome content accompanied by creation of multipolar spindles. The increased percentage of multinucleated cells in cells overexpressing DN-Nek7 or siRNA could support this option. According to this possibility the higher mitotic index observed in Nek7 siRNA overexpressing cells is the result of cells stuck late in mitosis.

The similar Nek6 and Nek7 kinases probably duplicated and diverged during early vertebrate evolution as the zebrafish genome harbors both Nek6 and Nek7 homologues, while only one protein exist in the databases of the early chordate, lancelet, and in echinoderms. It is not clear whether Nek6 and Nek7 retained similar or overlapping functions or whether they acquired different or even opposing functions. Supporting the last option is the observation that Nek6 and Nek7 respond differently to serum deprivation: Nek6 kinase activity falls down, while Nek7 activity is elevated [19]. In addition, the non-overlapping, tissue-specific, patterns of *nek6* and *nek7* mRNAs expression [4] could hint at different functions. Nek6 has been implicated in mitotic control by the elevation of its kinase activity during M phase, and by the requirement for its kinase activity for metaphase to anaphase transition [8]. However, no specific association of Nek6 protein to the centrosome, spindle or kinetochore has been found [8]. The present study, demonstrating spindle-related phenotypes and centrosomal localization of Nek7, could thus support distinct functions for Nek6 and Nek7.

As is the case for even bona fide centrosomal proteins [20–22], the centrosomal Nek7 fraction is quite small. It could suggest that Nek7 has additional non-centrosomal functions. Thus, even though depletion of the centrosomal fraction of Nek7 is the most probable explanation for the multipolar spindle phenotype of Nek7-siRNA treated cells, additional non-centrosomal effects should also be taken into account.

The majority of human cancer cells exhibit structural perturbation of the centrosome, supernumerary centrosomes and/or multipolar spindles. It is widely accepted that supernumerary centrosomes contribute to the formation of multipolar spindles and subsequently to defective chromosome segregation and to aneuploidy [23–25]. Misexpression of several members of the mitotic kinase families Aurora, Polo and Nek has been connected to centrosome misfunction and to tumorigenesis [26–28]. Further studies on Nek7 should shed light on the possible contribution of overexpression or depletion of this kinase to mammalian oncogenesis.

Acknowledgements: We thank Dr. Salisbury, Mayo Clinic, Rochester, for kindly providing Centrin-GFP expression vector. This study was supported by Grant No. 5508 from the Chief Scientist's Office of the Ministry of Health, Israel.

References

[1] O'Connell, M.J., Krien, M.J. and Hunter, T. (2003) Never say never. The NIMA-related protein kinases in mitotic control. Trends Cell Biol. 13, 221–228.

[2] Fry, A.M. and Nigg, E.A. (1995) Cell cycle. The NIMA kinase joins forces with Cdc2. Curr. Biol. 5, 1122–1125.
[3] Kandli, M., Feige, E., Chen, A., Kilfin, G. and Motro, B. (2000) Isolation and characterization of two evolutionarily conserved murine kinases (Nek6 and nek7) related to the fungal mitotic regulator, NIMA. Genomics 68, 187–196.
[4] Feige, E. and Motro, B. (2002) The related murine kinases, Nek6 and Nek7, display distinct patterns of expression. Mech. Dev. 110, 219–223.
[5] Roig, J., Mikhailov, A., Belham, C. and Avruch, J. (2002) Nercc1, a mammalian NIMA-family kinase, binds the Ran GTPase and regulates mitotic progression. Genes Dev. 16, 1640–1658.
[6] Roig, J., Groen, A., Caldwell, J. and Avruch, J. (2005) Active nercc1 protein kinase concentrates at centrosomes early in mitosis and is necessary for proper spindle assembly. Mol. Biol. Cell. 16, 4827–4840.
[7] Belham, C., Roig, J., Caldwell, J.A., Aoyama, Y., Kemp, B.E., Comb, M. and Avruch, J. (2003) A mitotic cascade of NIMA family kinases. Nercc1/Nek9 activates the Nek6 and Nek7 kinases. J. Biol. Chem. 278, 34897–34909.
[8] Yin, M.J., Shao, L., Voehringer, D., Smeal, T. and Jallal, B. (2003) The serine/threonine kinase Nek6 is required for cell cycle progression through mitosis. J. Biol. Chem. 278, 52454–52460.
[9] Miyake, S., Makimura, M., Kanegae, Y., Harada, S., Sato, Y., Takamori, K., Tokuda, C. and Saito, I. (1996) Efficient generation of recombinant adenoviruses using adenovirus DNA-terminal protein complex and a cosmid bearing the full-length virus genome. Proc. Natl. Acad. Sci. USA 93, 1320–1324.
[10] Graham, F.L. and van der Eb, A.J. (1973) A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology 52, 456–467.
[11] Zhou, C., Cunningham, L., Marcus, A.I., Li, Y. and Kahn, R.A. (2006) Arl2 and Arl3 regulate different microtubule-dependent processes. Mol. Biol. Cell 17, 2476–2487.
[12] White, R.A., Pan, Z. and Salisbury, J.L. (2000) GFP-centrin as a marker for centriole dynamics in living cells. Microsc. Res. Tech. 49, 451–457.
[13] Rieder, C.L. and Maiato, H. (2004) Stuck in division or passing through: what happens when cells cannot satisfy the spindle assembly checkpoint. Dev. Cell 7, 637–651.
[14] De Souza, C.P., Osmani, A.H., Wu, L.P., Spotts, J.L. and Osmani, S.A. (2000) Mitotic histone H3 phosphorylation by the NIMA kinase in Aspergillus nidulans. Cell 102, 293–302.
[15] Krien, M.J., West, R.R., John, U.P., Koniaras, K., McIntosh, J.R. and O'Connell, M.J. (2002) The fission yeast NIMA kinase Fin1p is required for spindle function and nuclear envelope integrity. EMBO J. 21, 1713–1722.
[16] Hinchcliffe, E.H., Li, C., Thompson, E.A., Maller, J.L. and Sluder, G. (1999) Requirement of Cdk2-cyclin E activity for repeated centrosome reproduction in Xenopus egg extracts. Science 283, 851–854.
[17] Lacey, K.R., Jackson, P.K. and Stearns, T. (1999) Cyclin-dependent kinase control of centrosome duplication. Proc. Natl. Acad. Sci. USA 96, 2817–2822.
[18] Okuda, M., Horn, H.F., Tarapore, P., Tokuyama, Y., Smulian, A.G., Chan, P.K., Knudsen, E.S., Hofmann, I.A., Snyder, J.D., Bove, K.E. and Fukasawa, K. (2000) Nucleophosmin/B23 is a target of CDK2/cyclin E in centrosome duplication. Cell 103, 127–140.
[19] Minoguchi, S., Minoguchi, M. and Yoshimura, A. (2003) Differential control of the NIMA-related kinases, Nek6 and Nek7, by serum stimulation. Biochem. Biophys. Res. Commun. 301, 899–906.
[20] Fry, A.M., Meraldi, P. and Nigg, E.A. (1998) A centrosomal function for the human Nek2 protein kinase, a member of the NIMA family of cell cycle regulators. EMBO J. 17, 470–481.
[21] Moudjou, M., Bordes, N., Paintrand, M. and Bornens, M. (1996) g-Tubulin in mammalian cells: the centrosomal and the cytosolic forms. J. Cell Sci. 109, 875–887.
[22] Paoletti, A., Moudjou, M., Paintrand, M., Salisbury, J.L. and Bornens, M. (1996) Most of centrin in animal cells is not centrosome-associated and centrosomal centrin is confined to the distal lumen of centrioles. J. Cell Sci. 109, 3089–3102.
[23] Boveri, T. (1914) Zur Frage der Enstehung Maligner Tumoren, Gustav Fisher Verlag, Jena.
[24] Salisbury, J.L., Whitehead, C.M., Lingle, W.L. and Barrett, S.L. (1999) Centrosomes and cancer. Biol. Cell 91, 451–460.
[25] Nigg, E.A. (2002) Centrosome aberrations: cause or consequence of cancer progression? Nat. Rev. Cancer 2, 815–825.
[26] Giet, R., Petretti, C. and Prigent, C. (2005) Aurora kinases, aneuploidy and cancer, a coincidence or a real link? Trends Cell Biol. 15, 241–250.
[27] Takai, N., Hamanaka, R., Yoshimatsu, J. and Miyakawa, I. (2005) Polo-like kinases (Plks) and cancer. Oncogene 24, 287–291.
[28] Hayward, D.G. and Fry, A.M. (2006) Nek2 kinase in chromosome instability and cancer. Cancer Lett. 237, 155–166.